HEAT-GELLING PROPERTIES OF RABBIT MYOFIBRILS : INFLUENCE OF MUSCLE TYPE

S. JOANDEL, C. TASSY, J. CULIOLI, A. OUALI

Station de Recherches sur la Viande, INRA Theix, 63122 Ceyrat, France

SUMMARY : The present work examined the relationship between muscle metabolic and contractile activities and the heat-gelling properties of extracted myofibrils. Metabolic and contractile types of 8 rabbit muscles were assessed by measuring citrate synthase (characterising the oxidative metabolic pathway), lactate dehydrogenase (characterising the glycolytic metabolic pathway) and myofibrilllar ATPase activities. Heat-gelling properties of myofibrils (20 mg/ml, in 0.6M KCl-0.04M potassium phosphate buffer, pH 6.0) were studied using a Carri-Med rheometer in the 30-80°C temperature range, using a heating rate of 1°C/min. As temperature raised from 30 to 80°C, two gelation peaks were observed for all muscles studied, the first one at 49-53°C and the second at 70-80°C. The temperature at which the gel strength attained its maximum value was muscle type dependent. The first maximum occurred at 53°C for SMp (type I) instead of 49°C for all the other muscles classified as type II. Similarly, the second transition started at 54°C for type II muscles and 58°C for type I muscle. Moreover, for both transitions, the storage modulus value (G') was highest for type II muscle than type I muscles. However significant differences in G' values were also observed between type II muscles suggesting a more complex relationship between muscle type and gelling properties than expected. Nevertheless, as checked by Scanning Electron Microscopy (SEM), differences in the gelling properties of myofibrils from various muscle types might be partly explained by differences in the ultrastructure of the gels.

1 - INTRODUCTION : Improvement of the commercial value of some bovine low grade muscles could be achieved by restructuration technology. These muscles are those with high collagen content and, hence, low tenderising capacities. Improvement of the texture of restructured meat products often requires the addition of texture additives having a high binding and gelling power. At the present time, these additives are all of non muscle origin (vegetable, dairy or plasma), leading to final products which cannot be labelled as 100% meat products. However meat by-products such as mechanically deboned meat are a source of proteins of high gelling capacity, mainly myofibrillar proteins, which could be incorporated in meat products in order to improve their texture. This aroused interest in the heat-gelling behaviour of myofibrillar proteins and in the variability noted between proteins extracted from different muscles of various animal species (EGELANDSDAL *et al.*, 1986 ; SANO *et al.*, 1990). These studies emphasized the influence of pH, ionic strength, protein concentration, as well as the effect muscle type on the heat-gelling properties of myofibrillar proteins (EGELANDSDAL *et al.*, 1986 ; SANO *et al.*, 1990). Large differences in gelling strength have thus been shown according to muscle types (FRETHEIM *et al.*, 1986 ; YOUNG *et al.*, 1991 ; ASHGAR *et al.*,1984). However, these investigations have been carried out on a restricted number of muscles (2 or 3) which have not been always clearly characterized. The present work intended therefore to determine the metabolic and contractile type of 8 rabbit muscles, and then to analyse the relationship with the heat-gelling properties of extracted myofibrils.

2 - MATERIAL AND METHODS :

2 - 1 Preparation of myofibrils and metabolic enzymes

Myofibrils were prepared from 8 rabbit muscles [*Psoas major* (PM), *Semimembranosus proprius* (SMp), *Supraspinatus* (SP), *Longissimus dorsi* (LD), *Semitendinosus* (ST), *Semimembranosus* (SM), *Pectoralis* (PE), *Triceps brachii* (TB)] excised just after slaughter. About 4 g of each muscle were homogeneised in a modified Ringer's solution (10 ml/g of wet muscle) (0.15 M NaCl, 0.025 M KCl, 3 mM $MgCl_2$, 1 mM PMSF and 4 mM EGTA) pH 6.5, using a Polytron. Collagen was eliminated by filtration on cheesecloth, after a 30 min extraction in ice. The homogenate was then centrifuged at 10 000 g for 15 min. The pellet of myofibrils was washed twice with a 0.05 M KCl solution (pH 6.5) containing 5 mM mercaptoethanol (10 ml/g of wet muscle). Part of the myofibrils pellet was suspended in this washing solution for ATPase activity assay. The other part was suspended in 0.6 M KCl-0.04 M potassium phosphate buffer pH 6.0 (buffer B) for rheological study and then dialysed for 12 hours at 2°C against the same buffer.

The metabolic enzyme extracts were obtained by homogeneising 1 g of each muscle in 20 ml of glycylglycine (63 mM) buffer pH 7.6 containing 500 mM sucrose, 6.2 mM EDTA, 125 mM NaF and 5 mM DTT with a Polytron by increasing gradually the speed to its maximum value. After a 1h extraction at 20° C, the homogenate was centrifuged at 5 000 g for 15 min and the supernatant used for citrate synthase (CS) and lactate dehydrogenase (LDH) activity measurement.

2 - 2 Enzymatic activities measurement

Myofibrillar ATPase activity was measured using the pH-Stat method previously described (OUALI and VALIN, 1981). Myofibrils (about 3

to 5 mg) were incubated in 174 mM KCl, 4 mM $MgCl_2$, 0.2 mM $CaCl_2$ and 4 mM ATP. The activity was expressed as µequivalent KOH/min/mg of proteins. Protein concentration was determined by the biuret method (GORNALL *et al.*, 1949) using bovine serum albumin as the standart.

2 - 2 - 2 Citrate synthase activity was assessed by a spectrophotometric method adapted from SRERE (1969) according to the following coupled reactions : SH-AcetylCoA + Oxalacetic acid (OAA) ----> Citrate + SH (1)

SH + DTNB ----> TNB-S-SCoA + TNB-SH (2)

0.1 ml of enzymatic extract diluted 20 fold in a physiological solution (9‰ NaCl) containing 0.15% bovine serum albumin (solution A) was incubated at 30 °C in 3 ml of 0.1 M Tris-HCl buffer pH 8 containing 2.5 mM EDTA, 7.5 mM S-Acetyl-Co A, 1 mM DTNB, and 10 mM OAA. The appearance of reduced thio-nitrobenzoic acid was followed spectrophotometrically at 412 nm. The activity was expressed as µmole/sec/g of wet muscle.

2 - 2- 3 Lactate dehydrogenase activity was measured by the spectrophototometric method of ANSAY (1974) according to the following reaction : pyruvate + $NADH+H^+$---> lactate + NAD^+

0.1 ml of enzymatic extract diluted 440 fold in the above solution A was incubated at 30°C in 3 ml of Triethanolamine buffer (50mM), pH 7.5, containing 10 mM $MgCl_2$, 5 mM EDTA, 0.3 mM NADH, 0.02% BSA, and 2.5 mM pyruvate. The disappearance of the NADH was followed spectrophotometrically at 340 nm. The activity was expressed as µmole/sec/g of wet muscle.

2 - 3 Rheological measurement

Heat-gelation of myofibrils (20 mg/ml in buffer B) were studied using a Carri-Med CSL 100 rheometer in oscillatory conditions. Myofibrils were put in the gap of a plate-plate cell (D=4 cm, e=0.8mm) and heated from 30°C to 80°C at a rate of 1°C/min . Rheological parameters, i.e. storage (G') and loss (G") moduli as well as loss tangent (∂), were determined after every 1°C increment.

2 - 4 Ultrastructural analysis

Gels were obtained by gradual heating (1°C) of myofibrillar extracts from 30°C up to 70°C. They were then fixed for 1h, at 4°C, in buffer B containing 3% of glutaraldehyde. After three washing in this buffer the gels were dehydrated using a series of alcohol (70°, 95°, 100°) and dried using the critical point method. The observation were carried out with a 505 Philips SEM at 15 KV.

3 - RESULTS AND DISCUSSION

3 -1 Classification of muscles

Myofibrillar ATPase activity values clearly show that rabbit muscles are mostly of fast-twitch type (Figures 1). Only the SMp muscle has significantly lower ATPase activity. When ATPase or LDH activity were plotted against CS activity, muscles are distributed within a triangle whose apices are defined by three different types of muscles namely PM, SP and SMp. According to PETTE and STARON (1990), muscles could be therefore classified in at least three categories :

1 - muscles having a high ATPase activity, and a relative high glycolytic activity and low oxydative activity. These muscles can be referred to as fast-twitch white or type IIB muscles. This group encompasses PM, ST, PE, SM and LD muscles.

2 - muscles having a low ATPase activity and a weakly glycolytic but strongly oxydative activities. These muscles can be referred to as slow-twitch red or type I. This group comprises only SMp muscle.

3 - muscles in which all three activities are high. These muscles can be referred to as fast-twitch red or type IIA muscles. This group comprises TB and SP muscles.

The oxydative capacity of type I muscles is thus intermediate between types IIB and IIA muscles. Similar muscle distribution has been observed in other animal species including guinea-pig (PETER *et al.*, 1972), sheep (BRIAND *et al.*, 1981), rat (BARNARD *et al.*, 1971) and horse (HOPPELER *et al.*, 1983). In pig (LABORDE, 1985) and cattle (ANSAY, 1974), muscles were distributed on a straight-line enabling discrimination of only two muscle types namely type IIB and type I.

3 - 2 Heat-gelation properties of myofibrils from different muscles

For all muscles investigated, rheograms reported in Figure 3 clearly show that heat-gelation of myofibrils is a two steps process. A simultaneous increase in G' and G" moduli starting at 40°C (46°C for SMp muscle) is first observed. G' shows a maximum value at 49°C for all muscles except SMp for which G' reached its maximum value at 54°C. At this stage, the gel is viscous for all muscles studied as attested by the fairly high value of ∂ (18-19°). In addition, maximum G' values were 3 to 4 fold lower for SMp and SP muscles than for PM

Moreover the temperatures at which the gelation process starts and reaches its maximum respectively were muscle type dependent. These results corroborate those of FRETHEIM *et al.* (1986) on bovine muscles showing that the starting temperature was lower for fast-twitch white muscles. The second step starts at 54-55°C (59-60°C for SMp, PE and SP). During this step G' increases while G" decreases. Then the rheological parameters seem to be stable beyond 70°C. At 80°C PM muscle shows the highest G' value followed by SM, TB, PE, and ST. Values for SMp and SP muscles are clearly much more lower than for the other muscles especially PM which exhibited a 2 to 3 fold higher G' value. On the basis of their metabolic and contractile type and their G' values at 70-80°C, four groups of muscles could be distinguished : (a) PM and SM muscles showing highest G' values and quite similar ATPase and LDH activities ; (b) TB, PE and SP muscles with slighly lower G' values and enzymes activities ; (c) LD and SP muscles which were characterised by a low G' value at both 49 and 80°C whereas all the above ones exhibited high G' values at 49° C. (d) SMp muscle which show high thermostability and give rise to gels of low rigidity. A similar muscle effect have been already reported in other animal species including bovine (FRETHEIM *et al.*, 1986 ; YOUNG *et al.*, 1991) and chicken (ASGHAR *et al.*, 1984). However the present finding would suggest that the possible original causes of the muscle are less simple than expected. The first question arising from the present findings is why muscles with closely related biochemical profiles, i.e. SM and LD on the one hand and TB and SP on the other hand, gave rise gels with significantly different strengths? In fact, this reflects very likely the complexity of muscle fibre type classification and, to a greater extent, of muscles which comprise various proportions of the different fibre types. Such oversimplified classification might be probably improved by considering myosin isoforms composition. At least four different heavy chain-based isomyosins have been thus described : one (Type I) for slow-twitch fibres and three (Types IIa, IIb and IId or IIx) for fast twitch fibres (PETTE and STARON, 1990). As myosin is responsible for the heat-gelating properties of myofibrils and assuming that myosin isoforms might show have different gelling capacities, muscle variability might be analysed more precisely.

Nevertheless, models such as rabbit are interesting since at least three types of muscles could be distinguished on the basis of enzymes activity ratios : the fast-twitch white muscles or type IIB, the slow-twitch red or type I muscles and the fast-twitch red muscle or type IIA.

3 - 3 Ultrastructure

Comparative SEM investigation revealed that gel ultrastructure vary between muscles. The comparison between LD and TB muscles clearly

Figure 1 : Rabbit muscles discrimination on enzymes activity ratios : myofibrillar ATPase (A) or lactate dehydrogenase (LDH) (B) versus citrate synthase (CS) activities. Each point is the mean value for five animals.
Muscles used are : *Psoas major* (PM), *Semimembranosus proprius* (SMp), *Supraspinatus* (SP), *Longissimus dorsi* (LD), *Semitendinosus* (ST), *Semimembranosus* (SM), *Pectoralis* (PE), *Triceps brachii* (TB).

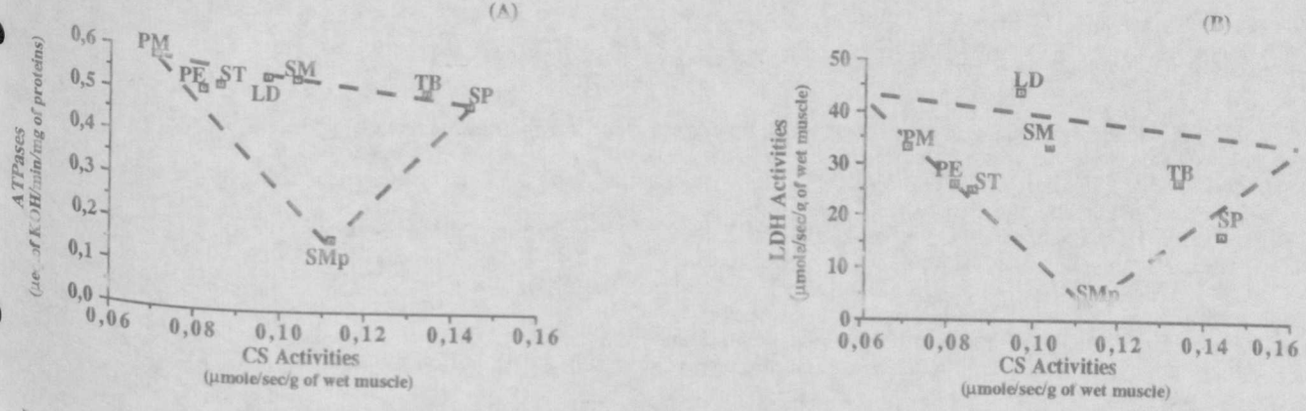
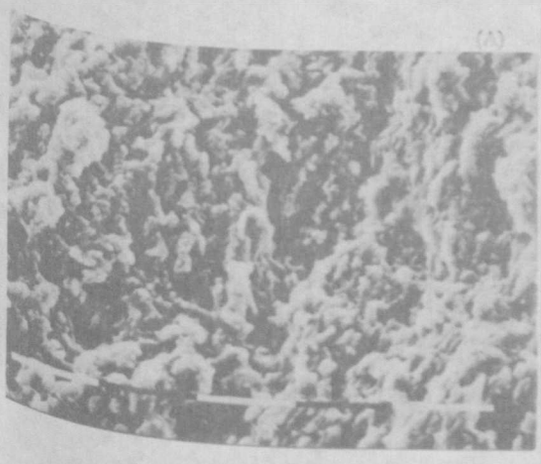


Figure 2 : SEM micrographs of myofibrils gels from LD (A) and TB (B) muscles. Bar = 10 µm



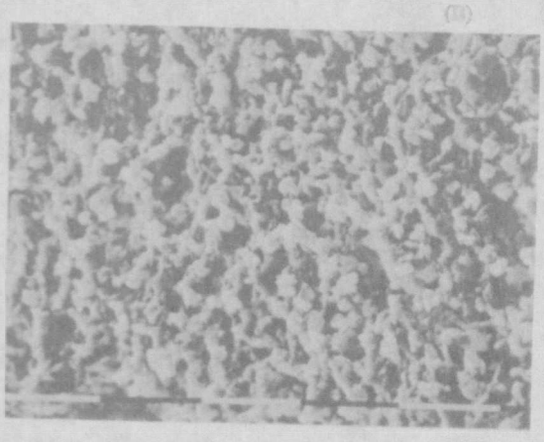
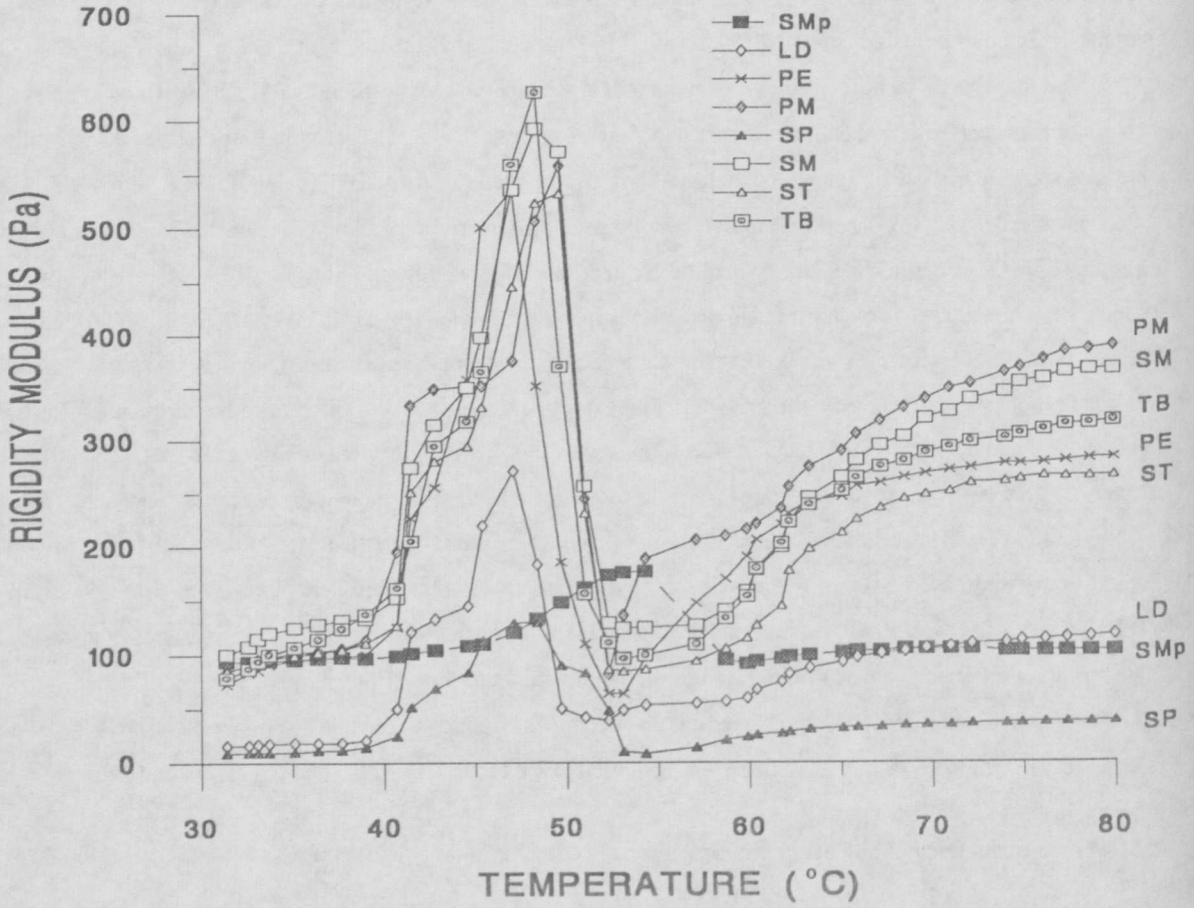


Figure 3 : Rheograms of different muscles.



showed that the network of LD myofibrillar proteins gels were made of finer filamentous structures and exhibited a higher density of these filaments than gels obtained with TB myofibrils (Figures 2). Hence the present data would suggest that fine stretched structures correspond to gels of greater rigidity a finding agreeing well with those previously reported by HERMANSSON (1986).

REFERENCES

ANSAY M., 1974. Individualité musculaire chez le bovin. Etude de l'équipement enzymatique de quelques muscles. Ann. Biol. Anim. Bioch. Biophy., 14, 471-486.

ASGHAR A., MORITA J.I., SAMEJIMA K. and YASUI T., 1984. Biochemical and functional characteristics of myosin from red and white muscles of chicken as influenced by nutritional stress. Agric. Biol. Chem., 48, 2217-2224.

BARNARD R.J., EDGERTON V.R., FURUKAWA T., PETER J.B.,1971. Histochemical, biochemical and contractile properties of red, white, and intermediate fibers. Am. J. Physiol., 220, 410-414.

BRIAND M., TALMANT A., BRIAND Y., DURAND R., 1981. Metabolic types of muscle in the sheep. I. Myosin ATPase, glycolytic, and mitochondrial enzyme activities. Eur. J. Appl. Physiol., 46, 347-358.

EGELANDSDAL B., FRETHEIM K. and SAMEJIMA K., 1986. Dynamic rheological measurements on heat-induced myosin gels. II. Differences in the gelation process depending on ionic strength : effects of protein concentration and addition of adenosine triphosphate or pyrophosphate. J. Sci. Food Agric., 37, 915-926.

FRETHEIM K., SAMAJIMA K. and EGELANDSDAL B., 1986. Myosins from red and white bovine muscles : Part 1-Gel strength (Elasticity) and water-holding. Capacity of heat-induced gels.Food Chem., 22, 107-121

GORNALL A., BARDAWILL C.J., DAVID M.M., 1949. Determination of serum proteins by means of biuret reaction. J. Biol. Chem., 177, 751-766.

HERMANSSON A.M., HARBITZ O., LANGTON M., 1986. Formation of two types of gels from bovine myosin. J. Sci. Food, Agric., 37, 69-84.

HOPPELER H., CLAASSEN H., HOWALD H., STRAUB R.,1983. Correlated histochemistry and morphometry in equine skeletal muscle. Snow D.H., Persson S.G.B., Rose R.I (eds). Equine exersice physiology, Granta, Cambridge, 184-192.

LABORDE D., TALMANT A., MONIN G., 1985. Activités enzymatiques métaboliques et contractiles de 30 muscles du Porc. Relations avec le pH ultime atteint après la mort. Reprod. Nutr. Develop., 25, 629-628.

OUALI A.,VALIN, 1981. Effect of lysosomal enzymes and Ca-ANP (Calcium Activated Neutral Proteinase) on the myofibrillar ATPase activities. Relationship with the ageing changes. Meat Science, 5, 233-245.

PETER J.B., BARNARD R.J., EDGERTON V.R., GILLESPIE C.A., STEMPEL K.E., 1972. Metabolic profiles of three fiber types of skeletal muscles in guinea pigs and rabbits. Biochem., 11, 2627-2633.

PETTE D., STARON R.S., 1990. Cellular and molecular diversities of mammalian skeletal muscle fibers. Rev. Physiol. Biochem. Pharmacol., 116, 1-76.

SANO T., NOGUCHI S.F., MATSUMOTO J.J., TSUCHIYA T., 1990. Effect of ionic strength on dynamic viscoelastic behavior of myosin during thermal gelation. J. Food Sci., 55, 51-54, 70.

SRERE P.A., 1969. Methods in Enzymology, 13, 11-16. Academic Press, New-York.

YOUNG O.A, TORLEY P.J. and REID D.H., 1991. Thermal Scanning Rheological properties of Myofibrillar Proteins from Muscles of Defined Fibre Type. Meat Science, 32, 45-63.